CLAIMS

WHAT IS CLAIMED IS:

1. A fluorogenic composition comprising a polypeptide backbone or a nucleic acid backbone joining two fluorophores of the same species whereby said fluorophores form an H-dimer resulting in quenching of the fluorescence of said fluorophores.

2. The fluorogenic composition of claim 1, wherein said composition comprises a polypeptide backbone.

3. The fluorogenic composition of claim 2, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 15 amino acids.

4. The fluorogenic composition of claim 2, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 8 amino acids.

5. The fluorogenic composition of claim 2, wherein said polypeptide backbone ranges in length from about 4 to about 31 amino acids.

6. The fluorogenic composition of claim 2, wherein said composition is attached to a solid support.

7. The fluorogenic composition of claim 2, wherein said composition is inside a mammalian cell.

8. The fluorogenic composition of claim 2, wherein said composition bears a hydrophobic group.

9. The fluorogenic composition of claim 8, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl

(Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

10. The composition of claim 9, wherein said hydrophobic group is Fmoc.

11. The composition of claim 9, wherein said hydrophobic group is Fa.

12. The composition of claim 9, wherein said hydrophobic group is attached to the amino terminus of the molecule.

13. The fluorogenic composition of claim 1, wherein said composition comprises a nucleic acid backbone.

14. The fluorogenic composition of claim 13, wherein said nucleic acid backbone comprises a restriction site.

15. The fluorogenic composition of claim 13, wherein said nucleic acid backbone is self-complementary and forms a hairpin.

16. The fluorogenic composition of claim 13, wherein said nucleic acid backbone ranges in length from about 10 to about 100 nucleotides.

17. The fluorogenic composition of claim 13, wherein said nucleic acid backbone ranges in length from about 15 to about 50 nucleotides.

18. The fluorogenic composition of claim 13, wherein said composition is attached to a solid support.

19. The fluorogenic composition of claim 13, wherein said composition is inside a mammalian cell.

20. The fluorogenic composition of claim 13, wherein said composition bears a hydrophobic group.

21. The fluorogenic composition of claim 20, wherein said hydrophobic group is selected from the group consisting of Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

22. The composition of claim 21, wherein said hydrophobic group is Fmoc.

23. The composition of claim 21, wherein said hydrophobic group is Fa.

24. The fluorogenic composition of claim 1, wherein said fluorophores are linked to the nucleic acid backbone or to the polypeptide backbone by linkers.

25. The fluorogenic composition of claim 1, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.

26. The fluorogenic composition of claim 1, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.

27. The fluorogenic composition of claim 1, wherein said fluorophores are carboxytetramethylrhodamine.

28. The fluorogenic composition of claim 1, wherein said fluorophores are carboxyrhodamine-X.

29. The fluorogenic composition of claim 1, wherein said fluorophores are carboxyrhodamine 110.

30. The fluorogenic composition of claim 1, wherein said fluorophores are diethylaminocoumarin.

31. The fluorogenic composition of claim 1, wherein said fluorophores are carbocyanine dyes.

32. A mammalian cell comprising a fluorogenic composition comprising a polypeptide backbone or a nucleic acid backbone joining two identical fluorophores whereby said fluorophores form an H-dimer resulting in the quenching of the fluorescence of said fluorophores.

33. The cell of claim 32, wherein said composition comprises a polypeptide backbone.

34. The cell of claim 32, wherein said composition comprises a nucleic acid backbone.

35. The cell of claim 32, wherein said composition bears a hydrophobic group.

36. The cell of claim 35, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

37. The cell of claim 36, wherein said hydrophobic group is Fmoc.

38. The cell of claim 32, wherein said hydrophobic group is Fa.

39. The cell of claim 32, wherein said hydrophobic group is attached to the amino terminus of the molecule.

40. The cell of claim 32, wherein said fluorophores are linked to the nucleic acid backbone or to the polypeptide backbone by linkers.

41. The cell of claim 32, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.

42. The cell of claim 32, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.

43. The cell of claim 42, wherein said fluorophores are carboxytetramethylrhodamine.

44. The cell of claim 42, wherein said fluorophores are carboxyrhodamine-X.

45. The cell of claim 42, wherein said fluorophores are carboxyrhodamine 110.

46. The cell of claim 42, wherein said fluorophores are diethylaminocoumarin.

47. The cell of claim 42, wherein said fluorophores are carbocyanine dyes.

48. A method of detecting the activity of a protease, said method comprising:

i) contacting said protease with a fluorogenic composition comprising a polypeptide backbone joining two fluorophores of the same species whereby said fluorophores form an H-dimer resulting in quenching of the fluorescence of said fluorophores; and

ii) detecting a change in fluorescence or absorbance of said fluorogenic composition where an increase in fluorescence or a change in absorbance indicates that said protease cleaves said polypeptide backbone.

49. The method of claim 48, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 15 amino acids.

50. The method of claim 48, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 8 amino acids.

51. The method of claim 48, wherein said composition is attached to a solid support.

52. The method of claim 48, wherein said composition is inside a mammalian cell.

53. The method of claim 48, wherein said composition is inside a insect cell.

54. The method of claim 48, wherein said composition is inside a yeast cell.

55. The method of claim 48, wherein said composition bears a hydrophobic group.

56. The method of claim 48, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

57. The method of claim 56, wherein said hydrophobic group is attached to the amino terminus of the molecule.

58. The method of claim 48, wherein said fluorophores are linked to the nucleic acid backbone or to the polypeptide backbone by linkers.

59. The method of claim 48, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.

60. The method of claim 48, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.

61. The method of claim 48, wherein said contacting is in a histological section.

62. The method of claim 48, wherein said contacting is in a cell culture.

63. The method of claim 48, wherein said contacting is contacting a seeded or cultured adherent cell.

64. The method of claim 48, wherein said contacting is in a cell suspension derived from a biological sample selected from the group consisting of a tissue, blood, urine, saliva, lymph, biopsy.

65. The method of claim 48, wherein said detecting is by a method selected from the group consisting of fluorescence microscopy, confocal microscopy, fluorescence microplate reader, flow cytometry, fluorometry, and absorption spectroscopy.

66. A method of detecting the activity of a nuclease or the presence of a nucleic acid, said method comprising:

i) contacting said nuclease or said nucleic acid with a fluorogenic composition comprising a nucleic acid backbone joining two fluorophores of the same species whereby said fluorophores form an H-dimer resulting in quenching of the fluorescence of said fluorophores; and

ii) detecting a change in fluorescence or absorbance of said fluorogenic composition where an increase in fluorescence or a change in absorbance indicates that said nuclease cleaves said nucleic acid backbone or that said nucleic acid hybridizes to said backbone.

67. The method of claim 66, wherein said nucleic acid backbone comprises a restriction site.

68. The method of claim 66, wherein said nucleic acid backbone is self-complementary and forms a hairpin.

69. The method of claim 66, wherein said nucleic acid backbone ranges in length from about 10 to about 100 nucleotides.

70. The method of claim 66, wherein said nucleic acid backbone ranges in length from about 15 to about 50 nucleotides.

71. The method of claim 66, wherein said composition is attached to a solid support.

72. The method of claim 66, wherein said composition is inside a mammalian cell.

73. The method of claim 66, wherein said composition is in solution.

74. The method of claim 66, wherein said composition bears a hydrophobic group.

75. The method of claim 74, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

76. The method of claim 66, wherein said fluorophores are linked to the nucleic acid backbone or to the polypeptide backbone by linkers.

77. The method of claim 66, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.

78. The method of claim 66, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.

79. The method of claim 66, wherein said contacting is in a histological section.

80. The method of claim 66, wherein said contacting is in a cell culture.

81. The method of claim 66, wherein said contacting is contacting a seeded or cultured adherent cell.

82. The method of claim 66, wherein said contacting is in a cell suspension derived from a biological sample selected from the group consisting of a tissue, blood, urine, saliva, lymph, biopsy.

83. The method of claim 66, wherein said detecting is by a method selected from the group consisting of fluorescence microscopy, confocal microscopy, fluorescence microplate reader, flow cytometry, fluorometry, and absorption spectroscopy.

84. A method of detecting the interaction of a first and a second molecule, said method comprising:

i) providing a first molecule having a first fluorophore attached thereto;

ii) providing a second molecule having a second fluorophore attached thereto wherein said first fluorophore and said second fluorophore are the same species of fluorophore and, when juxtaposed, form an H-dimer thereby quenching fluorescence produced by the fluorophores; and

iii) detecting a change in fluorescence or absorbance produced by said fluorophores where a decrease in fluorescence or a change in absorbance indicates that the first molecule and the second molecule are interacting.

85. The method of claim 84, wherein said first molecule and said second molecule are selected from the group consisting of a receptor and a receptor ligand, an antibody and an antigen, a lectin and a carbohydrate, and a nucleic acid and a nucleic acid binding protein.

86. The method of claim 84, wherein said fluorophore is linked to said first molecule by a linker.

87. The method of claim 84, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.

88. The method of claim 84, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.

89. A method of detecting a change in conformation or cleavage of a macromolecule, said method comprising:

i) providing a macromolecule having attached thereto two fluorophores of the same species whereby said fluorophores form an H-dimer resulting in quenching of the fluorescence of said fluorophores; and

ii) detecting a change in fluorescence or absorbance of said fluorophores wherein a change in fluorescence or absorbance indicates a change in conformation or cleavage of said macromolecule.

90. The method of claim 85, wherein said macromolecule is selected from the group consisting of a polypeptide, a nucleic acid, a lipid, a polysaccharide, and an oligosaccharide.

91. The method of claim 85, wherein said macromolecule is attached to a solid support.

92. The method of claim 85, wherein said macromolecule is inside a mammalian cell.

93. The method of claim 85, wherein said macromolecule bears a hydrophobic group.

94. The method of claim 93, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorenecarboxylic group, 9-florenecarboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh),Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimentyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO),t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

95. The method of claim 85, , wherein said fluorophores are linked to the macromolecule by linkers.

96. The method of claim 85, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.

97. The method of claim 85, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.

98. The method of claim 85, wherein said contacting is in a histological section.

99. The method of claim 85, wherein said contacting is in a cell culture.

100. The method of claim 85,, wherein said contacting is contacting a seeded or cultured adherent cell.

101. The method of claim 85,, wherein said contacting is in a cell suspension derived from a biological sample selected from the group consisting of a tissue, blood, urine, saliva, lymph, biopsy.

102. The method of claim 85, wherein said detecting is by a method selected from the group consisting of fluorescence microscopy, fluorescence microplate reader, flow cytometry, fluorometry, confocal microscopy, and absorption spectroscopy.